

Lagenidium sp. Ocular Infection Mimicking Ocular Pythiosis

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This is a report of a *Lagenidium* sp. in a Thai patient who was diagnosed with severe keratitis that was unresponsive to antibacterial and antifungal drugs. Examination of a corneal biopsy specimen confirmed the presence of aseptate hyphae. The internal transcribed spacer DNA sequence of the strain isolated showed 97% identity with *Lagenidium giganteum* and other *Lagenidium* species.

CASE REPORT

A 43-year-old Thai housewife was referred to King Chulalongkorn Memorial Hospital with lid swelling, pain, redness, and itching of the left eye for 3 weeks. Her clinical symptoms began 2 days after she flushed her eyes with tap water because of eye irritation during house cleaning. She was treated with systemic and topical antibiotics, but her condition worsened. The initial diagnosis was presumed to be a fungal corneal ulcer unresponsive to both topical and systemic antifungal agents. The patient had been given topical cefazolin, topical amikacin, topical natamycin, topical voriconazole, oral acyclovir, and oral itraconazole prior to transfer. Her medical history was unremarkable.

The initial examination revealed that the visual acuity of her left eye was finger counting at 1 foot. A slit lamp examination showed ciliary injection and a yellowish, midstromal, reticular-pattern corneal infiltration measuring 5 by 5.6 mm with a feathery edge (Fig. 1A). A corneal epithelial defect 4 by 3 mm in diameter

was detected over the infiltrated area. Posterior eye segment evaluation by ocular ultrasound demonstrated no sign of endophthalmitis. The right eye appeared normal.

In vivo confocal microscopy identified numerous branching fungus-like elements with interlocking and hyperrefractive thin lines in the corneal stroma (Fig. 1B). Direct examination of a left corneal scraping specimen revealed aseptate hyphae (Fig. 1C). A corneal biopsy specimen stained with hematoxylin and eosin and

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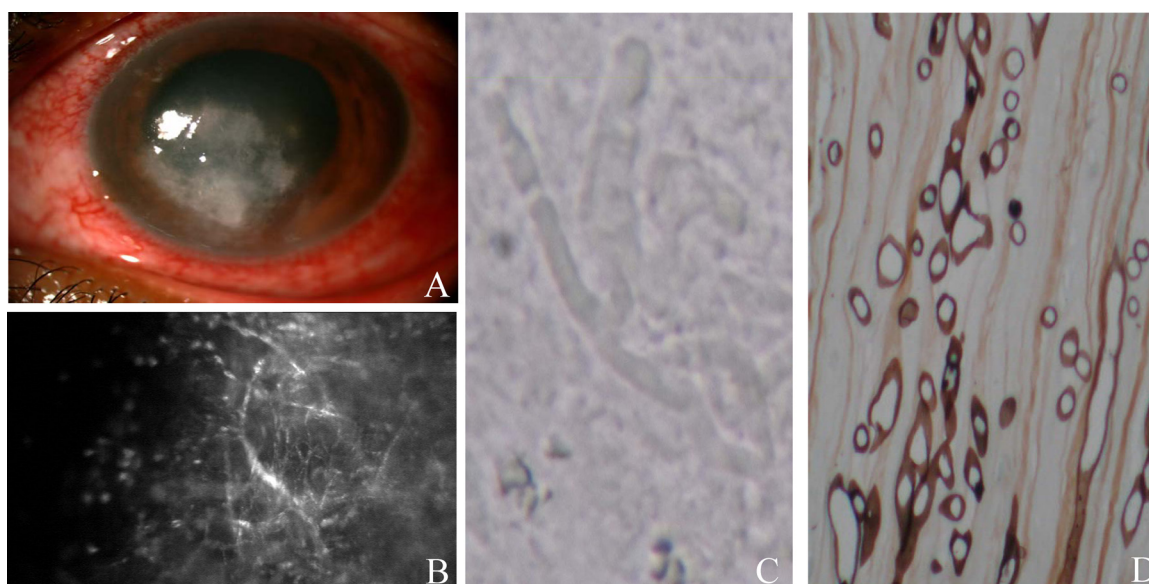


FIG 1 (A) Corneal infiltration with a reticular pattern and a feathery edge. (B) *In vivo* confocal microscopy showing numerous fungal elements as branching with interlocking and hyperrefractive thin lines in the corneal stroma. (C) Broad, rare, septate hyphae in a KOH preparation. Magnification, $\times 40$. (D) Longitudinal and transverse broad hyphae. GMS staining; magnification, $\times 40$.

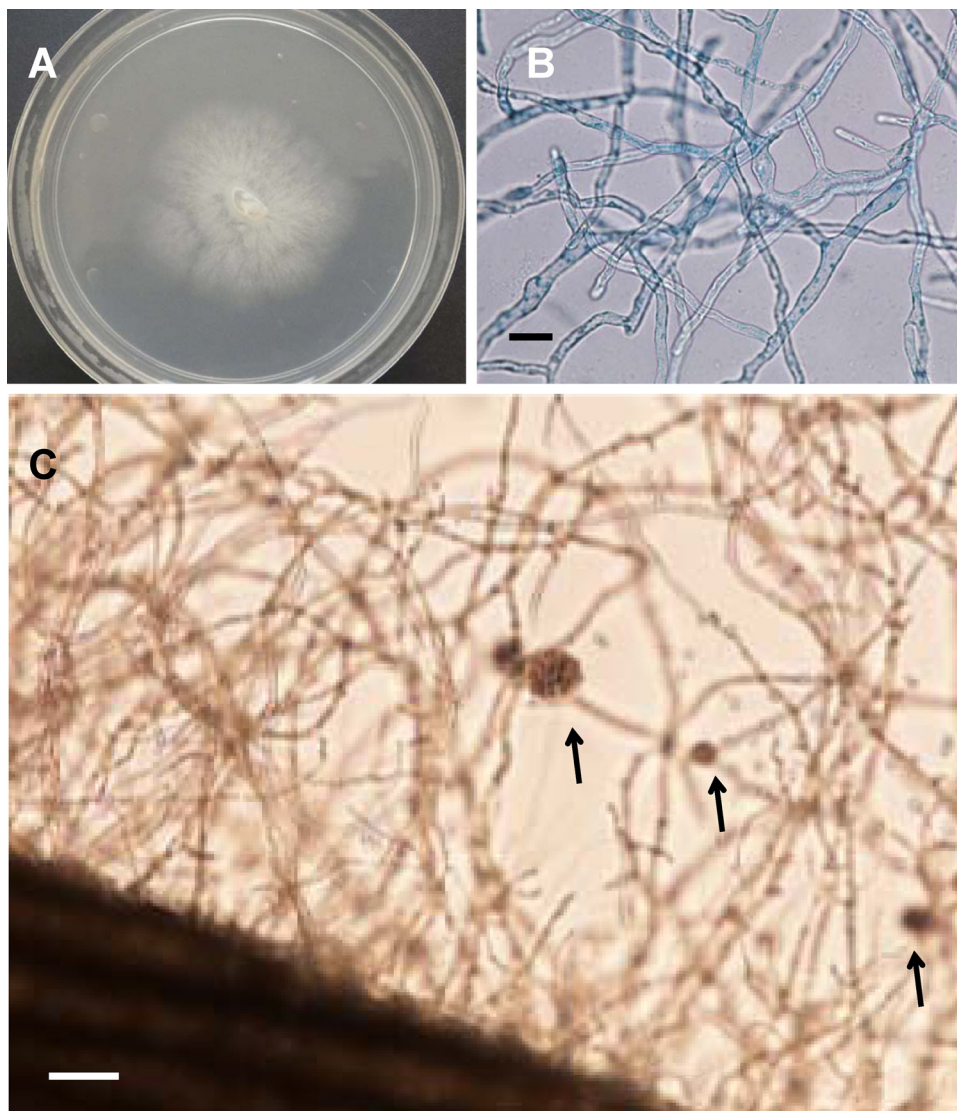


FIG 2 (A) A 48-h culture on 2% Sabouraud dextrose agar of the *Lagenidium* sp. recovered from the patient whose case is reported here. In this medium, the strain developed submerged glabrous white/yellowish colonies in less than 24 h. (B) Presence of broad rarely septate hyaline hyphae. Note that the strain does not develop fruiting bodies in this medium. Bar, 18 μ m. (C) Development of several sporangia with zoospores in liquid induction medium (arrows). Several swimming zoospores can be observed around the sporangia. Although the diameter of the *Lagenidium* hyphae from this case is greater, the hyphae have several phenotypic characteristics in common with *P. insidiosum*. Bar, 45 μ m.

Grocott's methenamine silver (GMS) stain showed longitudinal and transverse aseptate broad hyphae (Fig. 1D). Rapid growth of translucent submerged colonies was found in 2% dextrose Sabouraud agar at 37°C within 48 h (Fig. 2A). The strain was deposited at the American Type Culture Collection under accession number ATCC MYA-4932. The microscopic features of the strain recovered after 48 h of incubation at 37°C showed the development of broad, sparsely septate, hyaline, branched hyphae 9 to 15 μ m in diameter (Fig. 2B). Fruiting bodies could not be found on any of the 2% dextrose Sabouraud agar plates. Zoospores were induced in Sabouraud dextrose agar (pH 7.0) and corn meal agar (Difco) and then transferred to 2% water agar (pH 6.9) with boiled grass blades (1) (Fig. 2C). On the basis of these results and the relatively high frequency of ocular pythiosis in Thailand, *Pythium insidiosum* keratitis was suspected.

The patient received oral terbinafine, itraconazole, and topical

natamycin as antifungal agents. Immunotherapy with an in-house *Pythium* antigen was also used. This vaccine was modified from the original vaccine formulation (U.S. patents 5,948,413 and 6,287,573 B1) (2, 3). The treatment consisted of three subcutaneous injections of a 100- μ l (2-mg/ml) dose of antigen at 7-day intervals.

Internal transcribed spacer (ITS) amplicons, obtained by PCR using genomic DNA of the cultured strain and the clinical samples and universal primers ITS-1 and ITS-4 amplified PCR products smaller than those expected for the ITS sequences of *P. insidiosum* (4). The ITS amplicon was cloned into plasmid vector pCR 2.1-TOPO (Invitrogen, Carlsbad, CA), purified, and sequenced by BigDye Terminator chemistry in an ABI Prism 310 genetic analyzer (PerkinElmer, Foster City, CA). The sequence was then analyzed with the basic local alignment search tool available at the National Center for Biotechnology

Information. The analysis placed the isolated strain close to *L. giganteum* (97% identity) and to the other *Lagenidium* species available in the database and away from *P. insidiosum*. On the basis of this analysis, the strain was identified as a *Lagenidium* sp. (accession number JX646749).

Despite aggressive treatment with immunotherapy and topical and oral antifungal agents, the infection progressed rapidly. The additional two intracameral doses of amphotericin B were given 1 week apart. However, the ulcer worsened, showing thinning and impending perforation of the cornea. Therapeutic corneal transplantation was then performed. Despite the new approach, the infection recurred; thus, repeat penetrating keratoplasty (PK) was required. The infection was clinically controlled with no worsening visual acuity and no corneal infiltration. A follow-up examination 10 months after the second surgery showed no recurrence of the infection. A third surgical procedure to improve the patient's vision because of a scarred corneal graft was also scheduled.

The pathogenic oomycete *P. insidiosum* has been known as the only oomycete to infect mammals and birds (5–8). This notion was recently challenged by the finding of at least two different *Lagenidium* strains causing subcutaneous infections in cats and dogs (9–11). Grooters (11) introduced the term lagenidiosis to describe these infections along with a detailed description of their clinical, pathologic, and diagnostic features. However, a detailed description of the etiologic agents involved in this unusual infection has not been published. The lack of information on the taxonomy and phylogenetics of this novel group of pathogenic oomycetes was recently highlighted (12, 13).

So far, the infections caused by *Lagenidium* species have been reported only in lower animals (9–11). However, at least one unpublished human case was recently mentioned (14). We have had the opportunity to evaluate a case of human keratitis in Thailand caused by a filamentous oomycete that we have identified by molecular tools as a *Lagenidium* sp.

Although the diagnosis of *Lagenidium* keratitis was confirmed, the appropriate treatment to manage the infection could not be found in the literature. Brown et al. (9) reported the *in vitro* susceptibility of animal pathogenic *Lagenidium* sp. to terbinafine, caspofungin, and mefenoxam but not to itraconazole, posaconazole, and voriconazole. Also, it seems that a previous subcutaneous *Lagenidium* infection of a human patient responded only to posaconazole treatment (9). Other published data on the management of six *Lagenidium*-infected dogs could also provide additional information on the treatment of this infection in mammalian hosts; however, treatment in both studies did not produce satisfactory outcomes (10, 11).

Initially, our patient was treated with oral terbinafine, oral itraconazole, and topical natamycin as antifungal agents in combination with *P. insidiosum* vaccine, PK, and secondary PK. Systemic and topical antifungal agents were tapered in 3 months postoperatively, and the patient was discharged after 52 days. There has been no recurrence of the infection in the >10-month follow-up period. We could not conclude, however, whether adequate surgical removal of the affected tissue was the only factor in the suc-

cessful response. In conclusion, we report the first case of ocular lagenidiosis in a human successfully treated with the same management protocols used for ocular pythiosis (4, 5, 15, 16). The differentiation between *P. insidiosum* and *Lagenidium* spp. is required for appropriate management, since the latter pathogen is more aggressive, with a poor prognosis (9–11).

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